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EFFECTS OF NOSET-A ON RHESUS MONKEY VISUAL EVOKED RESPONSE AND SIDMAN AVOIDANCE TASK

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March 1977

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: AFRRI-SR77-1	2. GOVT ACCESSION NO. / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle): EFFECTS OF NOSET-A ON RHESUS MONKEY VISUAL EVOKED RESPONSE AND SIDMAN AVOIDANCE TASK	5. TYPE OF REPORT & PERIOD COVERED / 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): J. L. Mattsson, J. W. Crock, Jr. and L. J. Jenkins, Jr.* (*Navy Toxicology Unit, National Naval Medical Center)	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Armed Forces Radiobiology Research Institute (AFRRI) Defense Nuclear Agency Bethesda, Maryland 20014	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NWED QAXM A 905 13
11. CONTROLLING OFFICE NAME AND ADDRESS: Director Defense Nuclear Agency (DNA) Washington, D. C. 20305	12. REPORT DATE: March 1977 / 13. NUMBER OF PAGES: 16
14. MONITORING AGENCY NAME & ADDRESS (If different from Controlling Office)	15. SECURITY CLASS. (of this report): UNCLASSIFIED / 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

One male rhesus monkey (Macaca mulatta) was exposed to NOSET-A aerosol for 4 hours at a concentration of 2.4 ppm. Visual evoked response and Sidman avoidance task (free operant avoidance) data were collected after 2 hours and 4 hours of exposure. The visual evoked response was not affected, but a significant increase in response rate on the Sidman avoidance task occurred.

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SUMMARY
(Nontechnical)

NOSET-A contains triethylene glycol dinitrate, an organic dinitrate derived from nonvicinal diol, whereas the better known compounds, ethylene glycol dinitrate and propylene glycol dinitrate, are derived from nitration of vicinal diol. Both ethylene glycol dinitrate and propylene glycol dinitrate may cause severe debilitating headaches, vasodilatation, hypotension, and methemoglobin formation. In experimental animals, parenteral doses of NOSET-A also cause hypotension and methemoglobinemia. Unlike the vicinal dinitrates, NOSET-A causes emaciation and neurologic toxicity in chronically treated rabbits. Chronic exposure to vapor concentrations as low as 0.5 ppm has caused depression of a rhesus monkey's cued avoidance behavior.

In this experiment, one male rhesus monkey (Macaca mulatta) was exposed to NOSET-A aerosol for 4 hours at a concentration of 2.4 ppm. Visual evoked response and Sidman avoidance task (free operant avoidance) data were collected after 2 hours and again after 4 hours of exposure. The visual evoked response was not affected, but a significant increase in response rate on the Sidman avoidance task occurred. These data indicate that NOSET-A has neurobehavioral effects of potentially serious consequence, and that further testing, such as measurement of peripheral nerve conduction velocities and behavioral tests requiring a high degree of sensorimotor integration, is necessary.

PREFACE

This research was sponsored by the U. S. Naval Medical Research and Development Command. The chamber NOSET-A concentrations were controlled by R. A. Jones, and analyzed by L. Kurlansik. C. G. Franz and C. R. Curran were especially helpful in selection of the Sidman avoidance task for behavior control, and in assisting with the training schedule. Technicians B. A. Dennison and W. N. Fry were responsible for the day-to-day training and stabilization of the monkey, and operated the data collection equipment.

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INTRODUCTION

NOSET-A contains triethylene glycol dinitrate (TEGDN), an organic dinitrate derived from nonvicinal diol, whereas the better known compounds, ethylene glycol dinitrate and propylene glycol dinitrate, are derived from nitration of vicinal diol. Like ethylene glycol dinitrate and propylene glycol dinitrate, exposure to triethylene glycol dinitrate causes vasodilatation, hypotension, and methemoglobin formation.[3-5] In addition, TEGDN causes emaciation and neurologic toxicity in chronically treated rabbits. Acute NOSET-A intoxication leads to death from the combined effects of respiratory depression, tremoring and methemoglobinemia.[1] In a rhesus monkey, chronic exposure to NOSET-A vapor concentrations as low as 0.5 ppm caused depression of cued avoidance behavior.*

The visual evoked response (VER) has been utilized in toxicology and pharmacology to study the central nervous system effects of numerous chemicals including propylene glycol dinitrate,[10,15] carbon monoxide,[8] ethanol,[9,12] pentobarbital,[11] LSD and chlorpromazine,[2] and diazepam.[6] Because selected VER criteria can be objectively quantified and statistically evaluated, the VER was studied in the following NOSET-A experiment.

The natural VER variability due to change in level of arousal was minimized by having the monkey perform a shock avoidance task (Sidman) during the period the electroencephalogram (EEG) was collected for VER analysis. As a separate indicator of toxic effect, the Sidman avoidance task performance was monitored for deviations from normal response rate, and to detect incapacitation, should it occur.

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* Curran, C. R., Young, R. W., Franz, C. G., Middleton, G. R. and Jenkins, L. J., Jr. The effects of chronic inhalation of triethylene glycol dinitrate on conditioned avoidance behavior (in preparation)

METHODS

A 10-kg male rhesus monkey (Macaca mulatta) was exposed to NOSET-A aerosol at 2.4 ppm on two occasions, 1 week apart.

The Rochester-type inhalation exposure chamber, approximately 2 m^3 in volume, was modified for continuous use.[7] A strobe light source was located outside the chamber and behind the monkey. The chamber windows were covered with white cardboard to eliminate visual distraction of the monkey and to provide a diffuse reflective surface so that the light from the strobe would be uniformly distributed throughout the chamber, thereby reducing VER variability from changes in light intensity and direction of the animal's gaze. Air was passed through a gas washing bottle containing NOSET-A, delivering an aerosol into the chamber. To maintain aerosol concentrations at 2.4 ppm dilution, air was passed thr chamber at 0.5 to 1.0 m^3/min. The chamber atmosphere was m omatographically. Chamber atmosphere samples were drawn by va rough a seven-part automatic switching valve into a calibrated sample loop and then to a chromatograph equipped with a 6 ft x 1/4 in. (1.8 m x .6 cm) glass column containing 2.92 percent OV-17 on Anakrom Q 70/80 mesh. The system operated at a temperature of 110°C, using nitrogen at 70 ml/min and an electron capture detector at 150°C with a voltage of 20 V dc. Long lines and switching valves were heated to prevent condensation. A second method of analysis was to draw a known volume of the atmosphere through a bubbler equipped with a coarse frit and containing ethyl alcohol as the absorbing media. The sample was then read at 220 nm on a spectrophotometer (A = 1650).

Electroencephalogram (EEG), visual evoked response (VER) and Sidman avoidance task data were collected for 1 hour before the NOSET-A aerosol was introduced into the chamber (D_0) and again after 2 hours (D_2) and 4 hours (D_4) of exposure to the aerosol. This provided three 1-hour data periods. Control data were obtained the day before the exposure experiment.

The EEG was recorded from a single bipolar temporo-occipital lead. Electrodes were chronically implanted stainless screws machined to a "T" shape. They were implanted so that the top of the "T"s rested on the dura mater, and the bases were wired to an electrical plug affixed to the skull with dental acrylic.

The strobe flashed at a rate of 1/sec throughout the 1-hour data collection periods, and was off between periods. The EEG was amplified 1.2×10^4 times, band-pass filtered at 0.8 Hz to 90 Hz, and analyzed by a special purpose computer for visual evoked responses. Each individual VER was a composite of 100 samples, and there were 12 to 18 VERs collected during each data period. The filtered electroencephalogram was also recorded on paper for visual analysis.

Nonparametric Friedman and Wilcoxon statistical analysis was utilized.[14] To be considered statistically significant (1) the Friedman test had to show a significant variation among the six blocks of data (D_0, D_2, D_4) from the control day and day of exposure, and (2) the Wilcoxon test between data blocks had to be significant in two directions, the exposure data had to vary significantly from the control data of that day (e.g., D_0-D_2, exposure day), and the exposure data had to vary significantly from its paired control block of the preceding day (e.g., D_2 control-D_2 exposure).

Three wave form amplitudes of the VER were analyzed (designated A, B, and C waves). The A wave was positive at the occiput, had a latency of about 50 msec, and was measured base line to highest peak. The B wave was negative, had a latency of about 75 msec, and was measured peak to valley. The C wave was positive, had a latency of 100-200 msec, and was measured valley to highest peak (Figure 1).

Each 1-hour data period consisted of three 15-minute Sidman avoidance sessions (S^D), with a 5-minute rest period (S^Δ) between sessions. Visual evoked responses were collected only during Sidman avoidance sessions. The monkey could avoid a 0.2-sec shock indefinitely as long as the interval between

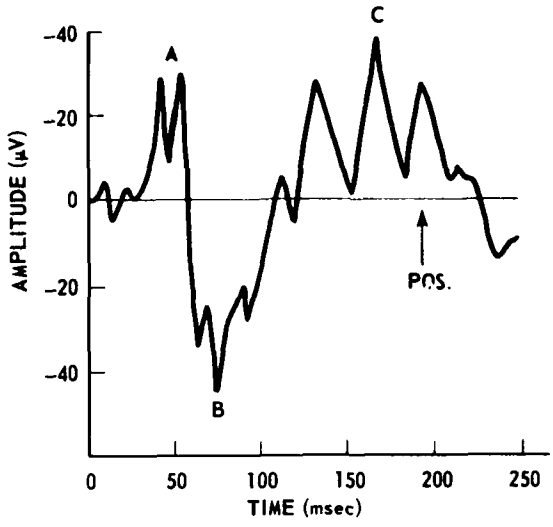

Figure 1. Visual evoked response from epidural bipolar temporo-occipital lead, occipital electrode positive

responses did not exceed 10 sec (response-shock interval 10 sec, shock-shock interval 1 sec). A cue light next to the response lever remained on continuously throughout the Sidman avoidance session. Sidman data were evaluated by one way analyses of variance. The control data (D_0, D_2, D_4 control day, D_0 exposure day) were first examined for homogeneity, then grouped and compared to the exposure data (D_2, D_4 exposure day).

RESULTS AND DISCUSSION

Exposure to 2.4 ppm NOSET-A aerosol for 2 to 4 hours caused no significant changes in electroencephalogram or any component of the visual evoked response, but the rate of Sidman avoidance responding was significantly increased (Figure 2). If NOSET-A had a diffuse effect throughout the brain at this concentration, the EEG or VER should have been affected. However, if the locus of action was specific, the effect might be missed by assessing only the visual system and the temporal and occipital cortex. It can be inferred, then, that the change in Sidman avoidance rate was not due to a diffuse change in the

central nervous system, but perhaps to a peripheral nervous system effect or to a psychologic change due to sensory detection of the aerosol.

In the Sidman avoidance task no exteroceptive stimulus warns the monkey of impending shock. When the shock occurs, it is so brief that the animal does not terminate the shock but rather postpones the next shock by pressing the lever. The motivating drive state appears to be anxiety, and the monkey

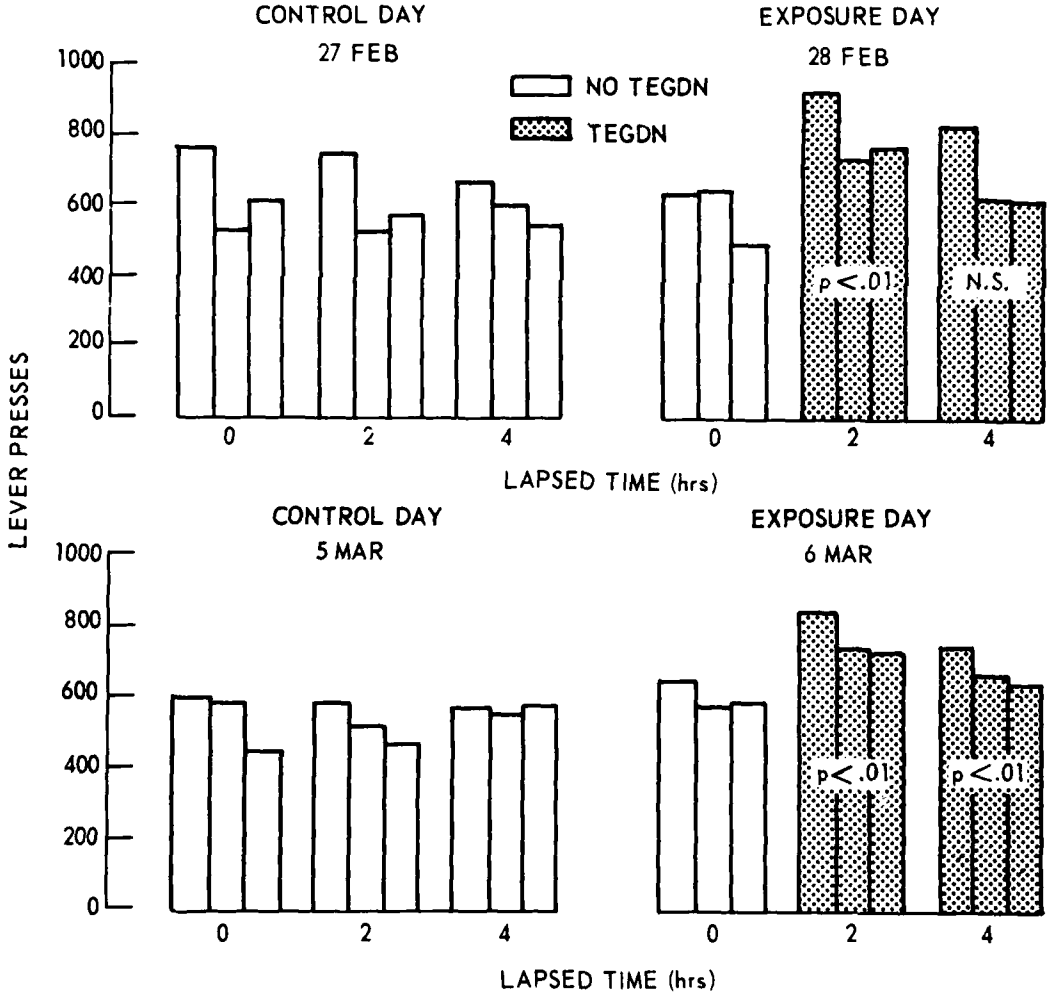

Figure 2. Sidman avoidance score. Each bar is total lever presses in 15-minute session, three sessions per 1-hour data block.

presses the lever to reduce the level of anxiety.[13] NOSET-A may increase the Sidman response rate by causing a centrally mediated change in anxiety level that is ameliorated by faster responding. Alternately, the anxiety level may have been increased by noxious effects of the aerosol such as eye or respiratory irritation, headache, or by other undefinable symptoms that may distress monkeys. This anxiety hypothesis is considerably weakened by the observation that NOSET-A had no effect on the VER, and anxiety and numerous other psychologic events are known to affect VERs.[16]

Andersen and Mehl[1] felt that NOSET-A has an effect on the peripheral nervous system, since it causes tremors in rats rather than tonoclonic convulsions, and a phrenic nerve-diaphragm preparation showed selective inhibition of the phrenic nerve but not the diaphragm. Intact rats were also hyperreactive to auditory and tactile stimuli.

The dose of NOSET-A causing gross motor tremor in rats is an order of magnitude greater than that causing the altered Sidman rate in the rhesus monkey. However, a subtle peripheral nervous system effect, coupled with hyperreactivity to sensory stimuli, would explain why task performance could change without significant changes occurring in the electroencephalogram and visual evoked response.

The results of this experiment tend to corroborate rather than refute the observations of Andersen and Mehl[1] and Curran et al.,* that NOSET-A has neurobehavioral effects of potentially serious consequence. The future focus of research might profitably include electrophysiological studies of peripheral nerve conduction and behavioral tests requiring a high degree of sensorimotor integration.

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* See footnote page 5

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